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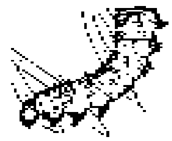

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EFFECTS OF PREPLANT SOIL TREATMENTS ON *FUSARIUM* AND *TRICHODERMA* POPULATIONS AND FUNGAL ROOT COLONIZATION OF 1-0 NONDISEASED PONDEROSA PINE SEEDLINGS – USDA FOREST SERVICE LUCKY PEAK NURSERY – BOISE, IDAHO

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ABSTRACT

Preplant soil treatments were implemented to determine effects on populations of potentially pathogenic *Fusarium* and potentially disease-suppressive *Trichoderma* spp. as well as root colonization by these and other selected fungi on healthy-appearing, bare root 1-0 ponderosa pine seedlings at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho. Soil treatments included fumigation with methyl bromide/chloropicrin (MBC), bare fallowing with periodic cultivation, and incorporation of two cultivars of winter *Brassica* cover crops. Soil *Fusarium* populations were greatly reduced by MBC fumigation; bare fallowing and incorporation of *Brassica* crops resulted in increased populations. *MBC fumigation and bare fallowing significantly decreased Trichoderma populations*. Incorporation of *Brassica* crops did not significantly affect soil *Trichoderma* populations. Nearly all sampled seedlings were infected with *Fusarium* species regardless of soil treatment. However, level of root colonization by these potentially pathogenic fungi was significantly reduced by pre-sowing MBC fumigation. High levels of root colonization by rhizosphere-inhabiting isolates of *Cylindrocarpon* and *Trichoderma* were common in some treatments. Assaying root colonization by selected fungi may supplement other parameters to evaluate effectiveness of soil treatments implemented to reduce pathogen populations and improve conifer seedling production.

INTRODUCTION

During recent years, efforts have been underway to develop alternatives to preplant soil fumigation for production of bare root seedlings in forest nurseries. These efforts have stemmed from the fact that methyl bromide, usually the most common and effective fumigant (James 1989), is a major potential destroyer of stratospheric ozone (World Meteorological Association 1995) and will no longer be available for non-emergency use in the United States after 2005 (James et al. 1994b; Stone et al. 1995, 1997). During investigations concerning alternative soil treatments, various parameters have been used to evaluate treatment efficacy. Such factors as soil populations of potential plant pathogens (*Fusarium* and *Pythium*), potential disease-suppressive fungi (*Trichoderma*), seedling density, disease, height, diameter and root biomass have all been measured (James et al. 1994b; Stone et al. 1995, 1997). Several of these parameters may more closely reflect associated factors rather than treatment effects on seedling production. For example, seedling density may often be more related to seedling size (and vice versa) than to treatment effects, especially by the end of the second growing season in bare root nurseries.

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Disease is not always a reliable indicator because of the non-uniform distribution of soil pathogens within fields and effects of environmental conditions on disease symptom expression (Bloomberg 1976; James and Beall 1999, 2000; James et al. 1991). Treatment effects on soil populations of *Fusarium* and *Pythium* spp. should give a rough estimate of efficacy. However, such populations may not necessarily reflect seedling disease potential because assayed populations include both pathogenic and non-pathogenic fungal strains which are often morphologically similar.

A potentially good indicator of treatment efficacy should be root biomass. If seedlings are more adversely affected by soilborne pathogens, their roots are more readily attacked, decay is increased, and root growth and overall biomass is reduced. However, in bare root seedling production, roots are routinely pruned prior to lifting to encourage increased fibrous root growth and to make extraction from soil easier with less root damage. Under such conditions, it is difficult to properly compare root systems because pruning effects compound possible interactions with root pathogens.

Another possible way to evaluate soil treatment efficacy is to monitor root infection by organisms targeted in preplant treatments. Since *Fusarium* spp. are usually the major pathogens of concern in most bare root forest nurseries in western North America (Bloomberg 1976; Bloomberg and Lock 1972; James et al. 1991), determining extent of seedling root infection by these fungi may indirectly give an accurate estimate of soil treatment efficacy.

This report compares populations of *Fusarium* (potential pathogens) and *Trichoderma* (potential antagonists) before and after preplant soil treatments and documents how treatments affected first-year seedling root infection by these fungi.

MATERIALS AND METHODS

Soil treatments were applied during the growing season prior to planting. Four treatments were conducted on plots that were replicated five times; plots were established within a production field in a complete randomized block design. The four treatments were: 1 = standard soil fumigation with methyl bromide and chloropicrin (MC-33) at 350 lbs./acre (392 kg/ha); 2 = bare fallow for the year prior to sowing with periodic cultivation to keep weed populations low and turn soil; 3 = cultivation of the Dwarf Essex variety of *Brassica* (as a winter crop: sown in the fall, grown during the fall and early spring, incorporated into soil at least 14 days prior to sowing); 4 = cultivation of a mustard variety of *Brassica* (grown and incorporated similar to Dwarf Essex). In the spring following soil treatments, one seedlot of ponderosa pine (*Pinus ponderosa* Laws.) was sown using standard nursery practices within all plots.

Prior to treatments, soil samples were collected from the tested field in a systematic manner. Fifty-four samples were collected along transects running through the field; 12-15 samples were collected from each of the four different treatment areas. Following treatments, just prior to sowing, six soil samples were collected from each treatment area. At each sample point a soil core was taken to a depth of about 8 in. (20 cm). Soil was placed in plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard soil dilutions (James and Beall 1999, 2000; James et al. 1996) were conducted to estimate populations of *Fusarium* and *Trichoderma*. Soil from each sample was initially sieved (2mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven-dried at about 100^{o}C for at least 24 hrs. until sample weight had stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For assays of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3 % water agar and thoroughly mixed. One ml of the solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly. Plates were incubated 5-7 days at about 24^{o}C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were expressed as colony-forming units (cfu) per g of

oven-dried soil. Selected *Fusarium* isolates were transferred to potato dextrose (PDA) and carnation leaf agar (Fisher et al. 1982) for identification using the taxonomy of Nelson et al. (1983). For each treatment, pre-treatment populations were statistically compared with pre-sowing populations using paired T Tests. Populations among the four treatments were compared with an analysis of variance; significantly different ($P \leq 0.05$) means were located using Tukey's HSD.

At the end of the first growing season (October), healthy-appearing seedlings were extracted from treatment plots. A total of 30 seedlings were randomly selected from each treatment. Seedlings were carefully extracted from soil to include as much of their root system as possible and refrigerated during transport to the laboratory. Seedling roots were washed thoroughly under tap water to remove soil particles, dissected into pieces about 5 mm in length, and surface sterilized in bleach (0.525% aqueous sodium hypochlorite). Ten root pieces were randomly selected from each seedling, rinsed in sterile water and placed on the *Fusarium* medium. Plates with roots were incubated as described above. Emerging fungi were identified to genus and selected *Fusarium* isolates were identified to species as described above. Percent of seedlings infected by particular fungi was calculated as "infection"; percent of sampled root pieces (10 per seedling) colonized by particular fungi was calculated as "colonization." Average percent colonization by different groups of fungi were compared statistically with an analysis of variance. Significantly different ($P \leq 0.05$) means were located using Tukey's HSD. All percentages underwent arc-sin conversion prior to analysis.

RESULTS AND DISCUSSION

Soil populations of *Fusarium* were significantly reduced by MBC fumigation (table 1). All other treatments resulted in significantly greater *Fusarium* populations. The least level of increase occurred in the fallowed fields. However, populations in plots with incorporated *Brassica* cover crops, exhibited a many fold increase in *Fusarium* (increases from over 800 to 1200%). *Trichoderma* populations were significantly reduced in fumigated and bare fallowed plots. The Brassica crop residues did not significantly affect populations of these fungi. Comparing the four treatments, it was quite evident that MBC fumigation most severely reduced *Fusarium* populations. Pre-sowing populations in fallowed plots were significantly less than those incorporated with *Brassica* residues and were below putative disease threshold levels (James and Beall 1999, 2000; James et al. 1996).

Four *Fusarium* species were isolated from nursery soil (table 2). By far the most common species was *F. oxysporum* Schlect.; it comprised more than 98% of the *Fusarium* population sampled both before and after treatments. Two morphologically-distinct isolates (morphotypes) of *F. oxysporum* were routinely isolated from soil. These were differentiated primarily on their typical colony morphology on both Komada's medium and PDA. Although both colony types exhibited profuse aerial mycelium, hyphal texture and pigmentation were consistently different between the two morphotypes. This cultural diversity may not necessarily be related to pathogenic potential, but was useful in segregating these two morphotypes. Three other *Fusarium* species were isolated at very low levels. These included *F. sporotrichioides* Sherb., *F. acuminatum* Ell. & Ev., and *F. equiseti* (Corda) Sacc.

Fusarium spp. were isolated from all sampled seedlings grown in soil that was either bare fallowed or incorporated with *Brassica* cover crops (table 3). Almost all seedlings grown in MBC fumigated soil were likewise infected by *Fusarium* spp., although level of root colonization was significantly less than seedlings from the other three treatments (table 4). Extensive root colonization by *Fusarium* spp. was evident on many sampled seedlings even though these seedlings appeared healthy and did not display either above- or below-ground disease symptoms.

Level of root colonization by *Cylindrocarpon* spp., which are common inhabitants of plant rhizospheres (Booth 1966) and usually either

Table 1. Effects of preplant treatments on soil populations of *Fusarium* and *Trichoderma* – USDA Forest Service Lucky Peak Nursery, Boise, Idaho.[1]

Treatment[2]	Replication	*Fusarium*		*Trichoderma*	
		Pretreat	Presow	Pretreat	Presow
1	1	168	0	4,176	671
	2	452	34	2,563	371
	3	486	0	3,214	1,212
	Average	340.7a	11.2a*	3,317.6a	751.2a*
	% Change	-96.7		-77.4	
2	1	349	1,004	2,775	1,606
	2	954	706	3,031	1,847
	3	202	807	4,297	1,479
	Average	489.8a	838.8b*	3,321.9a	1,644.3ab*
	% Change	+712.2		-50.5	
3	1	1,015	3,424	1,863	2,920
	2	469	19,485	2,853	1,408
	3	1,366	4232	2,735	6,827
	Average	945.1b	9,047.8d*	2,527.9a	3,739.0c
	% Change	+857.3		+47.9	
4	1	242	1,108	3,131	2,417
	2	442	9,629	2,141	3,118
	3	188	607	3,178	1,651
	Average	290.5a	3,781.0c*	2,816.7a	2,395.5b
	% Change	+1,201.5		-14.9	

[1] Values in table are colony-forming units per gram of oven-dry soil. Pre-sowing averages followed by an asterisk are significantly different (P≤0.05) using a paired T Test. Percent change reflects changes from pre-treatment to pre-sowing population counts.
[2] 1 = fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; 4 = cultivation and incorporation of mustard variety of *Brassica*. Comparing average population counts for each group of fungi among the four treatments, means followed by the same letters are not significantly different (P≤0.05) using Tukey's HSD.

Table 2. *Fusarium* isolated from soil undergoing presowing treatments – USDA Forest Service Lucky Peak Nursery, Boise, Idaho.

Fungal Isolates[2]	Treatment[3]				
	1	2	3	4	All Treatments
FOXY 1					
Pretreat	54.8	42.1	54.5	32.4	48.4
Presow	0	31.0	38.3	26.0	34.4
FOXY 2					
Pretreat	43.6	56.8	44.5	64.6	50.4
Presow	100.0	64.8	60.5	72.5	64.1
All FOXY					
Pretreat	98.4	98.9	99.0	97.0	98.8
Presow	100.0	95.8	98.8	98.5	98.5

Table 2, continued

Fungal Isolates[2]	Treatment[3]				
	1	2	3	4	All Treatments
FSPO					
Pretreat	0	1.0	1.0	1.5	0.7
Presow	0	0	0	0	0
FACU					
Pretreat	1.6	0	0	1.5	0.5
Presow	0	0	0	0	0
FEQU					
Pretreat	0	0	0	0	0
Presow	0	4.2	1.2	1.5	1.5

[1] Numbers in table are percent of *Fusarium* isolates recovered from soil samples that were within the appropriate species.
[2] FOXY= *Fusarium oxysporum* (1 and 2 delineate different morphotypes); FSPO = *F. sporotrichioides*; FACU = *F. acuminatum*; FEQU = *F. equiseti*.
[3] 1 = fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; 4 = cultivation and incorporation of mustard variety of *Brassica*.

Table 3. Effects of preplant soil treatments on infection of roots of nondiseased 1-0 ponderosa pine seedlings by *Fusarium* and other selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho.[1]

Fungal Species[2]	Treatments[3]			
	1	2	3	4
FOXY 1	57	100	100	100
FOXY 2	83	97	100	97
FAVE	0	0	37	20
FACU	10	7	0	0
FSPO	3	0	7	0
FSCR	0	0	0	10
FPRO	0	3	0	0
FSOL	0	0	0	3
All *Fusarium*	93	100	100	100
Cylindrocarpon	13	83	23	80
Trichoderma	97	70	90	80
Penicillium	7	0	7	7
Other Fungi	30	17	20	7

[1] Values in table are percent of seedlings sampled (30 per treatment) infected by appropriate fungus.
[2] FOXY = *F. oxysporum* (1 and 2 refers to morphologically-different isolates) FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSPO = *F. sporotrichioides*; FSCR = *F. scirpi* var. *compactum*; FPRO = *F. proliferatum*; FSOL = *F. solani*.
[3] 1 = fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; 4 = cultivation and incorporation of mustard variety of *Brassica*.

either saprophytes or very weak pathogens (Booth 1966; Dumroese et al. 2000; James et al. 1994a) was significantly lower in MBC-treated plots and those with incorporated Dwarf Essex *Brassica* crops (table 4). These fungi apparently were very sensitive to MBC fumigation and did not increase significantly when Dwarf Essex plant residues were incorporated into soil.

Interestingly, levels of root colonization by *Trichoderma* spp., common soil saprophytes with the potential to induce pathogen suppressiveness (Papavizas 1985), were significantly higher in MBC-treated plots compared to all others. Previous work (James and Beall 1999; Vaartaja 1967) had shown that *Trichoderma* spp. were often initial colonizers of chemically fumigated soil. It is important that these organisms colonize fumigated soil before potential pathogens to induce disease suppression.

By far the most commonly isolated *Fusarium* species from seedling roots was *F. oxysporum*. The same two morphotypes of *F. oxysporum* recovered from nursery soil were routinely isolated from seedling roots. This species is a very common soil inhabitant in forest nurseries and often causes serious seedling diseases (Bloomberg 1976; James et al. 1989a, 1991). *Fusarium oxysporum* is a common colonizer of root cortical tissues in both healthy-appearing and diseased seedlings (Bloomberg 1976; James and Gilligan 1988a, 1988b). When it initiates root tissue deterioration and necrosis, the fungus acts as a pathogen. However, some isolates colonize root tissues without eliciting host disease responses and thus act like non-

Table 4. Effects of preplant soil treatments on colonization of roots of nondiseased 1-0 ponderosa pine seedlings by *Fusarium* and other selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho.[1]

Fungal Species[2]	Treatments[3]			
	1	2	3	4
FOXY 1	17.7	67.3	78.3	76.3
FOXY 2	28.0	46.0	68.0	56.7
FAVE	0	0	6.3	3.0
FACU	1.7	1.3	0	0
FSPO	0.3	0	1.3	0
FSCR	0	0	0	2.0
FPRO	0	0.7	0	0
FSOL	0	0	0	0.7
All *Fusarium*	45.7a	93.0b	99.3b	97.0b
Cylindrocarpon	3.0a	48.0b	8.7a	38.3b
Trichoderma	68.7b	21.0a	33.7a	28.0a
Penicillium	0.7a	0a	0.7a	0.7a
Other Fungi	7.3b	4.7ab	2.3a	0.7a

[1] Values in table are percent of root pieces sampled (10 per seedling; 30 seedlings per treatment) infected by appropriate fungus.
[2] FOXY = *F. oxysporum* (1 and 2 refers to morphologically-different isolates) FAVE = *F. avenaceum*; FACU = *F. acuminatum*; FSPO = *F. sporotrichioides*; FSCR = *F. scirpi* var. *compactum*; FPRO = *F. proliferatum*; FSOL = *F. solani*.
[3] 1 = fumigation with methyl bromide/chloropicrin; 2 = bare fallow with periodic cultivation; 3 = cultivation and incorporation of Dwarf Essex variety of *Brassica*; 4 = cultivation and incorporation of mustard variety of *Brassica*. For each fungus, means followed by the same letter are not significantly different (P=0.05) using Tukey's HSD. Percentages underwent arc-sin transformation prior to analysis.

pathogenic endophytes (Dumroese et al. 1993; Gordon and Martyn 1997; Gordon and Okamoto 1992a). Extent of root colonization by this fungus (not necessarily associated with disease symptom production) is a good indicator of overall soil populations because *F. oxysporum* so readily colonizes plant root tissues (Dumroese et al. 1993; Gordon and Martyn 1997; James et al. 1991; Smith 1967).

Several other *Fusarium* species were also isolated from seedling roots, including *F. acuminatum, F. avenaceum* (Fr.) Sacc., *F. sambucinum* Fuckel, *F. sporotrichioides, F. culmorum* (W.G. Smith) Sacc., and *F. solani* (Mart.) Appel & Wollenw. Some of these species may include isolates that are pathogenic on conifer seedlings (James 2000, James and Perez 2000; James et al. 1989a, 1989b, 1991), but many are common soilborne saprophytes on seedling roots.

Characterizing the *Fusarium* population within forest nursery soils is difficult without implementing molecular methods, which may often differentiate pathogenic from non-pathogenic isolates (Appel and Gordon 1995; Gordon and Okamota 1992a, 199b, 1992c). Standard soil and root assays only reveal presence of particular species, but do not predict disease potential. Either molecular characterization or elaborate pathogenicity tests are required to determine level of disease potential within particular *Fusarium* populations (James 2000; James and Perez 2000; James et al. 1989b, 2000).

The primary goal of nursery soil treatment is to reduce potential pathogen populations to levels where diseases do not adversely affect seedling production or quality (Stone et al. 1995, 1997). Unless target organisms are capable of infecting seedling root tissues, they will not be able to initiate disease. Therefore, determining level of root infection by potential pathogens may be more effective in evaluating treatment efficacy than typical soil assays.

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